



THE UNIVERSITY  
of NORTH CAROLINA  
at CHAPEL HILL

VAN HECKE-WETTACH  
CAMPUS BOX 3380  
CHAPEL HILL, NC 27599-3380

RECEIVED

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BUCHANAN INGERSOLL, P.C.  
T 919.962.1234  
F 919.962.1277  
[www.law.unc.edu](http://www.law.unc.edu)

July 22, 2004

Re: U.S. Patent Application No. 10/700118  
Ramon Eritja et al., "Target sequences for the detection  
of the west nile virus," Attorney Docket No. 030570

Ramon Eritja, Inventor  
c/o Duane A. Stewart III, Esq.  
Buchanan Ingersoll PC  
One Oxford Centre, 20th Floor  
301 Grant Street  
Pittsburgh PA 15219

Dear Mr. Eritja:

I am writing to call your attention to a printed publication that may constitute material prior art with respect to the above-referenced patent application.

Enclosed please find a copy of a CD-ROM document entitled "On the preparation and utilization of isolated and purified oligonucleotides," which I produced on March 9, 2002 and contributed to the public collection of the Kathrine R. Everett Law Library of the University of North Carolina on March 14, 2002.

For your convenience, I have also enclosed a hard copy of the initial portion of the text file stored on that CD-ROM. As you can ascertain from that excerpt, the CD-ROM reference contains a full written description of several million oligonucleotides of between 8 and 12 nucleotides in length inclusive, together with methods of making and using each.

I believe that the reference is material prior art at least with respect to one or more claims of the above-referenced application. Accordingly, I would recommend that the attorney or agent handling this application promptly disclose this reference to the Patent Office. As a courtesy, I would appreciate a written acknowledgement that he or she has done so.

If you wish to discuss this matter, I can be reached at the above phone number or by email at [chin@unc.edu](mailto:chin@unc.edu).

Sincerely yours,

Andrew Chin  
Associate Professor

## On the Preparation and Utilization of Isolated and Purified Oligonucleotides

Andrew Chin

The University of North Carolina School of Law

March 9, 2002

The term "isolated" as used herein refers to a nucleotide sequence that has been manually produced and is separated from its native, *in vivo*, cellular environment and is present in the substantial absence of other biological molecules of the same type. The term "purified" as used herein for nucleotide sequences preferably means lacking significant quantities of other biological macromolecules of the same type (but water, buffers, and other small molecules, can be present).

### Preparation of Isolated and Purified Oligonucleotides

As described in U.S. Patent No. 5,808,022 (issued Sept. 15, 1998) (William D. Huse), oligonucleotide synthesis proceeds via linear coupling of individual monomers in a stepwise reaction. The reactions are generally performed on a solid phase support by first coupling the 3' end of the first monomer to the support. The second monomer is added to the 5' end of the first monomer in a condensation reaction to yield a dinucleotide coupled to the solid support. At the end of each coupling reaction, the by-products and unreacted, free monomers are washed away so that the starting material for the next round of synthesis is the pure oligonucleotide attached to the support. In this reaction scheme, the stepwise addition of individual monomers to a single, growing end of an oligonucleotide ensures accurate synthesis of the desired sequence. Moreover, unwanted side reactions are eliminated, such as the condensation of two oligonucleotides, resulting in high product yields.

Oligonucleotides are constructed by conventional procedures such as those described in J. Sambrook et al., *Molecular Cloning: A Laboratory Manual* 10.42-46 (3rd ed. 2001); K. Itakura et al., *Synthesis and Use of Synthetic Oligonucleotides*, 53 *Ann. Rev. Biochemistry* 323 (1984); M.D. Matteucci & M.H. Caruthers, *Synthesis of Deoxynucleotides on a Polymer Support*, 103 *J. Am. Chem. Soc'y* 3185 (1981); S.A. Narang, *DNA Synthesis*, 39 *Tetrahedron* 3 (1983). Oligonucleotide chains up to about 70 nucleotide residues long are preferably synthesized on automated synthesizers well known in the art (such as the Beckman Oligo 1000 or the Applied Biosystems ABI 392 DNA Synthesizer). Present-day DNA synthesizers are so efficient that oligonucleotides up to about 25 nucleotides in length generally do not contain significant quantities of truncated DNA fragments and hence do not require purification by gel electrophoresis. If necessary, however, purification of synthetic oligonucleotides can be achieved by one of several methods, as described in J. Sambrook, *supra*, at 10.48-49; including denaturing polyacrylamide gel electrophoresis, as described in J. Sambrook, *supra*, at 10.11-16; T. Atkinson & M. Smith, *Solid-Phase Synthesis of Oligodeoxyribonucleotides by the Phosphate-Triester Method*, in *Oligonucleotide Synthesis: A Practical Approach* 35-82 (M.J. Gait ed. 1984).

### Utilization of Oligonucleotides

As described in U.S. Patent No. 6,316,191 (issued Nov. 13, 2001) (Radoje T. Drmanac), hybridization depends on the pairing of complementary bases in nucleic acids and is a specific tool useful for the general recognition of informational polymers. Diverse research problems using hybridization of a synthetic oligonucleotide of known sequence include, amongst others, the different techniques of identification of specific clones from cDNA and genomic libraries, detecting single base pair polymorphisms in DNA, generation of mutations by oligonucleotide mutagenesis, and the amplification of nucleic acids in vitro from a single sperm, an extinct organism, or a single virus infecting a single cell.

Synthetic oligonucleotides of arbitrary nucleotide sequence are utilized in biological research, wherein oligonucleotides of specified length and random nucleotide sequence are synthesized using known procedures such as those described in Huse, *supra*; U.S. Patent No. 5,639,595 (issued June 17, 1997) (Christopher K. Mirabelli et al.). Arbitrary oligonucleotide primers of specified length may be used in the synthesis of cDNA probes from mRNA as described in Sambrook, *supra*, at 9.38-40; J.G. Williams et al., DNA Polymorphisms Amplified By Arbitrary Primers Are Useful As Genetic Markers, 18 Nucleic Acids Research 6531 (1990), in the systematic evolution of ligands by exponential enrichment as described in U.S. Patent No. 6,331,398 (issued Dec. 18, 2001) (Larry Gold & Craig Tuerk); C. Tuerk & L. Gold, Systematic Evolution of High-Affinity RNA Ligands of Bacteriophage T4 DNA Polymerase in Vitro, 249 Science 505 (1990), and in sequencing by hybridization as described in Drmanac, *supra*. Preferably, oligonucleotide primers and probes are characterized by sequences of 8 to 20 nucleotides that have moderate G+C content, are free of homopolymeric runs and directly or inversely repeated regions.

The disclosures of all publications and patents set forth hereinbefore are expressly incorporated herein by reference.

### Sequence Listing

The listing of sequences set forth hereinafter consists of all sequences of 8 to 12 nucleotides that have between 40 and 60 percent G+C content and are free of homopolymeric runs of 4 or more bases and directly or inversely repeated regions of 4 or more bases. Based on the disclosures herein and the knowledge of a person of ordinary skill in the art, it will be apparent to such a person how to make and use an isolated and/or purified oligonucleotide characterized by any of the following nucleotide sequences: